Histologic analyses of flapless ridge preservation in sockets with buccal dehiscence defects using two alloplastic bone graft substitutes

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Abstract: OBJECTIVES To investigate whether one of two synthetic bone substitute materials used for ridge preservation in the extraction sockets with buccal dehiscence defects was superior regarding new bone formation and ridge preservation and to compare it to sites left for spontaneous healing. MATERIALS AND METHODS In sixteen dogs, P3 and P4 were hemi-sectioned and the respective distal roots were extracted. Following the preparation of a mucoperiosteal flap without vertical releasing incisions, 50% of the buccal bone was carefully removed. The extraction sites were randomly assigned either to a ridge preservation procedure (alloplastic bone substitute material (two test groups)) or to spontaneous healing (control group). Descriptive histology and histomorphometric analyses were performed at healing times of 4, 8, and 16 weeks. In case of homogeneous variances, the results were analyzed by one-way ANOVA, followed by Tukey's post-hoc test. If inhomogeneous, the data was analyzed using Welch-type ANOVA, followed by the Games-Howell post-hoc test. RESULTS The use of bone substitute material led to significantly greater horizontal dimensions amounting to 3.3 mm (SD = 0.67; test 1) and 3.5 mm (SD = 0.72; test 2) compared to spontaneous healing (1.7 mm, SD = 0.23) at 16 weeks of healing ($p < 0.0001$). A significant difference was observed between spontaneous healing and the test groups in terms of newly formed bone tissue at 4, 8, and 16 weeks ($p = 0.001$), with values reaching 7.9, 21.8, and 36.8% (test 1), 5.0, 10.4, and 29% (test 2), and 26.2, 43.5, and 56.4% (control), but there were no significant differences between the test groups ($p > 0.05$). The final ridge profile was more favorable after ridge preservation ($p < 0.001$) as demonstrated by a loss of 28.8% (spontaneous healing) and an increase in both test groups at 16 weeks (test 1 = 60.5% and test 2 = 31.2%). CONCLUSIONS The use of alloplastic materials rendered greater horizontal dimensions and a more favorable maintenance of the ridge profile. CLINICAL RELEVANCE Alloplastic bone substitute materials can successfully be used for ridge preservation procedures.

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Abstract

Objectives: To investigate whether one of two synthetic bone substitute materials used for ridge preservation in extraction sockets with buccal dehiscence defects was superior regarding new bone formation and ridge preservation and to compare it to sites left for spontaneous healing.

Materials and methods: In sixteen dogs P3 and P4 were hemi-sectioned and the respective distal roots extracted. Following the preparation of a mucoperiosteal flap without vertical releasing incisions, 50% of the buccal bone was carefully removed. The extraction sites were randomly assigned to either a ridge preservation procedure (alloplastic bone substitute material (two test groups)) or to spontaneous healing (control group). Descriptive histology and histomorphometric analyses were performed at healing times of 4, 8 and 16 weeks. In case of homogeneous variances, the results were analyzed by one-way ANOVA followed by Tukey's post-hoc test. If inhomogeneous, the data was analyzed using Welch-type ANOVA, followed by the Games-Howell post-hoc test.

Results: The use of bone substitute material led to significantly greater horizontal dimensions amounting to 3.3mm (SD=0.67; test1) and 3.5mm (SD=0.72; test2) compared to spontaneous healing (1.7mm, SD=0.23) at 16 weeks of healing ($p<0.0001$). A significant difference was observed between spontaneous healing and the test groups in terms of newly formed bone tissue at 4, 8 and 16 weeks ($p=0.001$) with values reaching 7.9%, 21.8%, 36.8% (test1); 5.0%, 10.4%, 29% (test2); 26.2%, 43.5%, 56.4% (control), but no significant differences between the test groups ($p>0.05$). The final ridge profile was more favorable after ridge preservation ($p<0.001$) as demonstrated by a loss of 28.8% (spontaneous healing) and an increase in both test groups at 16 weeks (test1 = 60.5% and test2 = 31.2%).

Conclusions: The use of alloplastic materials rendered greater horizontal dimensions and a more favorable maintenance of the ridge profile.

Clinical relevance: Alloplastic bone substitute materials can successfully be used for ridge preservation procedures.

Introduction

Following tooth extraction, alveolar bone is subject to remodeling and resorption processes (Schropp et al., 2003, Araujo et al., 2015b). In case of spontaneous healing, the alveolar ridge presents with a pronounced loss in alveolar bone volume, mainly at the buccal aspect. The width of the alveolar ridge may thus be reduced by up to 50% during the period of 12 months after tooth extraction with approximately two thirds of this loss occurring within the first 3 months (Araujo and Lindhe, 2005, Schropp et al., 2003).

In order to delay or limit bone resorption following tooth extraction, the concept of alveolar ridge preservation has been suggested and widely investigated (Avila-Ortiz et al., 2014). After atraumatic extraction, flapless alveolar ridge preservation (RP) can be performed at the time of tooth extraction. For that purpose, a bone substitute material is placed in the socket and covered by a membrane, thus maintaining the ridge profile and promoting the formation of new bone (Araujo et al., 2015b).

Results of a clinical study demonstrated that the ridge width in the aesthetic area was reduced by 25% when subjected to spontaneous healing versus a reduction of only 3% in sites grafted with a xenograft (Araujo et al., 2015a). These results were confirmed by a systematic review addressing the effect of ridge preservation compared to spontaneous healing. Ridge preservation turned out to be effective in limiting physiologic ridge reduction in non-molar teeth (Avila-Ortiz et al., 2014).

A variety of materials were investigated with outcomes from preclinical and clinical studies being inconsistent (Atieh et al., 2015, Jambhekar et al., 2015). More recently, a number of alloplastic materials were developed to serve as bone graft substitutes. These materials are inert, osseo-conductive and serve as scaffolds for new bone formation (Rolvien et al., 2018). Results from a recent clinical study on ridge preservation procedures showed similar outcomes in terms of volume preservation based on histologic and radiologic outcomes for both xenogeneic and alloplastic bone substitute materials (Mardas et al., 2010, Mardas et al., 2011). The same outcomes regarding new bone formation were reported by two recent systematic reviews (Danesh-Sani et al., 2017, Papageorgiou et al., 2016). Only limited data exists in terms of alloplastic bone substitute materials as investigated in the present study (Valdivia-Gandur et al.,

2016, Wildburger et al., 2017, Favero et al., 2016). Some volumetric data were published (Naenni et al., 2017), but only limited histologic data on alloplastic materials used for ridge preservation (Kakar et al., 2017). However, data on the preclinical use of non-resorbable (ePTFE)-membranes compared to a resorbable alloplastic membrane showed comparable results (Al Salamah et al., 2012). This study has been designed as a preclinical trial to improve the current level of evidence and understanding of the behavior of alloplastic materials when used for ridge preservation procedures. As there still is a lack of information regarding the behavior and integration of alloplastic materials both the histologic and volumetric outcomes were evaluated and compared to the so-called 'gold-standard', the xenogeneic bone substitute material.

Thus, the aim of the present study was to histologically investigate the effect of ridge preservation using two different alloplastic bone graft substitutes in combination with a collagen membrane compared to sites left for spontaneous healing.

Materials and methods

Study design

This study was performed with sixteen adult male beagle dogs (more than 1-year-old, weighing between 10 to 20 kg). The study was drafted as a randomized controlled experimental study and performed according to the ARRIVE guidelines (Kilkenny et al., 2011). All animals were kept in a purpose-designed room and fed a soft diet. The protocol was approved by the local ethical committee of NAMSA (Lyon, France) and the study was conducted in accordance with the OECD Good Laboratory Practice regulations, ENV/MC/CHEM (98)17, with the European Good Laboratory Practice regulations, 2004/10/EC Directive and with the United States Food and Drug Administration Good Laboratory Practice regulations, 21 CFR 58.

Extractions and ridge preservation

Detailed description of the procedures and the medication were described in a previous publication reporting on the same study subjects (Naenni et al., 2017). In brief, three calibrated

surgeons performed bilateral hemi-sections and extractions of the distal roots of mandibular teeth P3 and P4 without raising a flap (Fig. 1a). The remaining mesial roots were root canal treated according to an established protocol (Thoma et al., 2010). After hemisection and extraction, a mucoperiosteal flap was prepared both on the lingual and buccal side extending to the apical portion of the extraction socket (vertically) and with a mesio-distal dimension exceeding the extraction socket by 2mm, but without a vertical releasing incision. Subsequently, 50% of the buccal bone was carefully removed using a bur and without rupturing the soft tissues (Fig. 1a). A total number of 64 extraction sites (four sites in 16 dogs) were originally planned and randomly assigned to one of the three treatment groups:

- Test group 1: in situ hardening alloplastic bone substitute material (biphasic calcium phosphate particles consisting of 60% hydroxyapatite (HA) and 40% beta-tricalcium phosphate (ß-TCP) coated with poly lactic-co-glycolic acid (PLGA)); (GUIDOR *easy-graft* CRYSTAL, Sunstar Suisse SA, Etoy, Switzerland) + collagen membrane (Jason Membrane, Botiss Biomaterials GmbH, Zossen, Germany).
- Test group 2: alloplastic bone substitute material (biphasic calcium phosphate consisting of 60% HA and 40% ß-TCP; (Straumann® BoneCeramic, Institut Straumann AG, Basel, Switzerland) + collagen membrane (Jason Membrane, Botiss Biomaterials GmbH, Zossen, Germany).
- Control group: negative control, blood clot.

One dog showed no P4 in both sides of the mandible (due to a genetic abnormality that was detected just before the surgery). After the study, it was decided to exclude all sites from this dog from the final evaluation. Therefore a total of 60 extraction sites instead of the operated 62 sites were assessed.

In both test groups, the extraction sites were filled with the respective allograft according to the manufacturer's instruction for use. The sites were filled up to the level of the bone crest without overfilling the buccal contour (Fig. 1b). In order to cover the bone graft material and to prevent from epithelial ingrowth, a collagen membrane was placed underneath the buccal and lingual mucoperiosteal flap. The control sites were left for spontaneous healing without further

placement of a biomaterial. In all groups, a horizontal mattress suture was applied at each site in order to obtain partial wound closure. After a healing phase of 13 to 14 days, sutures were removed. Soft tissue healing was assessed at the time of suture removal and wound closure was rated according to three categories: closed (fully keratinized); partially open (partially keratinized); fully open (not keratinized).

Sacrifice

The dogs were sacrificed 4 (n=5), 8 (n=5) and 16 (n=6) weeks after ridge preservation using an overdose of pentobarbital (60 mg/kg/i.v., Dolethal; Vetoquinol, France) after sedation with tiletamine-zolazepam (25 mg/kg, IM, Zoletil®100, Virbac, Carros, France).

Histologic preparation

After fixation in neutral buffered formalin, the hemi-mandibles were dissected into individual blocks with a band saw (one block per site). After complete fixation, the specimens were dehydrated in alcohol solutions of increasing concentration, cleared in xylene and embedded in PMMA. One central section per site (bucco-lingual) was prepared using the EXAKT microcutting system (EXAKT Technologies Inc., Oklahoma City, USA). The histologic slides were stained with a modified polychromatic stain (Paragon) allowing for qualitative, semi-quantitative and quantitative histopathologic analyses. All analyses were performed by an experienced NAMSA-histologist using a microscope (Eclipse 80i, Nikon, Minato, Tokyo, Japan) coupled with a digital camera (DS-Fi1 NIKON).

Quantitative Evaluation of the Ridge Dimension

A region of interest (ROI 1) corresponding to the new hard tissue contour consisting of newly formed bone (for the control group) and bone substitute material and newly formed bone (for the bone tissue; test groups) was drawn (Fig.2). Changes in horizontal (bucco-lingual) width of the ridge were determined at two different levels within ROI 1. Parallel lines to the horizontal plane were placed at 1 mm and 3 mm below the lingual bone crest, representing two different levels of the alveolar ridge. The horizontal length along these lines ranged from the defect

margin (old bone) to the newly formed bone (for the control group) or/and to the new bone and/or bone substitute margin (for both test groups). Measurements were expressed in micrometers [μm] for: newly formed bone, bone substitute material and soft tissue. A second region of interest (ROI 2) was drawn corresponding to the estimated original ridge profile (area equivalent to 100% of the ridge) (Fig.3). The difference between the estimated and the true ridge profile (ROI 2 – ROI 1) was calculated and expressed in [%].

Statistical analysis

The statistical analysis was conducted using an parametric and non-parametric mixed model analyses (Software SAS version 9.4, SAS inc.).The groups were compared with post hoc test in case of a significant result. A Bonferroni correction was applied for the multiple testing. The level of significance was set at 5%.

Results

After histological preparations of the sites, a total of 12/60 sites had to be excluded due to their quality or inappropriate sectioning. This included 2/21 sites from group Test 2, 7/21 sites group Test 1 and 3/18 sites for the sham-operated group (control).

Healing

At the time of suture removal 13 out of 62 sites presented with an incomplete wound closure (partially open/ partially keratinized) with a diameter of ≤1 mm (n=8, test1; n=4, test2; n=1, control), whereas the remaining sites had healed completely. Sites with a delayed healing were locally disinfected using a 0.5% chlorhexidine-spray (CooperPharma Limited, Delhi, India), but otherwise left untouched.

Descriptive histologic findings

At 4 weeks, moderate bone growth from both the apex of the defect and the lingual wall was noted in the control group. The newly formed woven bone was harboring a limited number of osteoblasts. The remaining defect area was filled with a vascularized fibro-connective tissue

infiltrated with macrophages. In both test groups, the defect sites were markedly invaded by vascularized, fibro-inflammatory tissue surrounding the granules. These tissues were infiltrated by macrophages and giant cells admixed with lymphocytes and plasma cells. Slight initial signs of cell-mediated superficial material degradation were noted in both test groups. However, over the period of the study no considerable degradation was visible and the bone substitute materials were partly osseointegrated. Along the adjacent native bone, scatters of newly formed premature bone were present. At 16 weeks, a more distinct crestal bone formation was observed resulting in pronounced vertical ridge augmentation in all three groups. A moderate to marked crestal bone growth was present in the control group shaping the defect. Both test groups showed newly formed bone and a moderate number or osteoblasts, whilst still exhibiting non-osseointegrated granules (Fig.6).

Histomorphometric analysis –-Evaluation of ridge profile (ROI 1)

The values for overall horizontal ridge width within the augmented area (ROI 1) (Fig.2) at 1 mm and 3 mm below the lingual bone crest are expressed in mm (Fig.4 & Table 1). At 4 weeks, the mean total horizontal width measured 2.6mm (SD=0.5;1mm) and 3.4mm (SD=0.4;3mm) for group test1, 3.2mm (SD=0.6;1mm) and 3.3mm (SD=0.4;3mm) for group test2 and 0.8mm (SD=0.7;1mm), respectively 1.5mm (SD=0.6;3mm) for the control group. The values for total horizontal width in the control group were statistically significantly lower at both levels ($p \leq 0.0001$) compared to the test groups. The values for horizontal bone width resulted in statistically significant differences between the control and the test groups ($p<0.0001$, 1mm; $p=0.0088$, 3mm).

At 8 weeks, the difference in overall mean horizontal between group test1 with 2.4mm (SD=0.3;1mm) and 2.7mm (SD=0.6;3mm) and group test2 with 3.0mm (SD=0.4;1mm) and 3.4mm (SD=0.7;3mm) was not significant at both levels. The control group exhibited a lower ridge width amounting to 1.1mm (SD=0.3;1mm) and 1.5mm (SD=0.7;3mm) and reached statistical significance ($p \leq 0.000$) compared to both test groups. The values for horizontal bone width resulted in statistically significant differences between the control and the test groups at 1mm ($p=0.0019$), but not at the 3mm level.

At 16 weeks, measurements revealed an overall mean ridge width of 3.0mm (SD=0.4;1mm); 3.3mm (SD=0.6;3mm) for group test1, 3.1mm (SD=0.6;1mm); 3.5mm (SD=0.7;3mm) for group test2 and 1.0mm (SD=0.4;1mm); 1.7mm (SD=0.1;3mm) for group control. The values for the control group were statistically significantly lower compared to both test groups at both levels ($p<0.005$). The values for horizontal bone width resulted in statistically significant differences between the control and the test groups this time at 3mm ($p<0.002$), but not at the 1mm level.

Histomorphometric analysis – Tissue Composition

The values for bone tissue (newly formed bone and bone substitute material) and soft tissue within the estimated ridge contour (ROI 2) were measured according to *Figure 3* and are reported in % (*Table 2).*

Over time, the amount of bone tissue increased in all groups. Values measured 6.6%, 17.2%; (SD=5.8%, 2.9%; test1); 3.6%, 7.8%; (SD=2.1%, 8.4%; test2); 50.7% 67.7%; (SD=8.7, 12.2; control) at 4 and 8 weeks, respectively. At 16 weeks, the amount of bone tissue reached 25.6% (SD=6.8%, test1); 24.9% (SD=12.8%, test2) and 77.0% (SD=9.8%, control). The difference was significant between the control group and both test groups at all time points ($p<0.005$). The difference between the test groups did not reach statistical significance at any time-point (Table 2).

Maintenance of Ridge Profile – ROI 1 / ROI 2

At 16 weeks, the comparison between the true and the estimated ridge profile (ROI 1 / ROI 2) revealed for the control group, that the initial ridge profile was reduced (mean maintenance of ridge profile - ROI 1/ROI 2: 70%, SD±0.10; Q1=0.74, Q3=0.75). For the grafted groups, the resulting ridge profile (ROI1) exceeded the estimated initial ridge profile (ROI2) result 158% (SD±30%; test 1) and 130% (SD±15%; test 2) of the original ridge. Positive values were obtained at all time-points in both test groups, whilst the control group showed a loss in ridge

profile ranging from 46% (4w) to 30% (16w). The differences between the test groups and the control group reached statistical significance at all time points (*p<0.005) *(Figure 5)*.

Discussion

The results of the present study illustrate that: i) the use of alloplastic bone substitute materials led to significantly greater horizontal ridge dimensions compared to the control group (spontaneous healing); ii) bone tissue was observed to a similar extent in both test groups at all time points; iii) the control group rendered a significantly higher amount of bone tissue compared to the test groups at all time points; iv) the ridge profile was significantly better maintained in the test groups compared to the control group.

Ridge preservation procedures aim at limiting bone resorption and remodeling processes after tooth extraction. Thus, bone substitute materials are applied at the extraction site in order to better maintain the ridge contour. There is broad clinical evidence reporting on the outcomes of ridge preservation (RP) procedures (Araujo et al., 2015b, Avila-Ortiz et al., 2014). Numerous pre-clinical trials investigated xenogeneic bone substitute materials (Araujo et al., 2015b, Jung et al., 2017, Araujo and Lindhe, 2009, Araujo et al., 2008). However, the use of alloplastic materials is far less investigated (Mardas et al., 2010, Mardas et al., 2011, Gholami et al., 2012)**.** Only limited data exists regarding histological outcomes for alloplastic bone substitute materials used for ridge preservation procedures. Pre-clinical (Cardaropoli et al., 2005) and clinical data (Gholami et al., 2012, Mardas et al., 2010) have investigated both Tri-Calcium-Phosphate- (TCP) and Hydroxyl-Apatite- (HA)-containing materials and their behavior in extraction sockets. In general, the available evidence reports similar results regarding maintenance of the ridge and histological findings for alloplastic materials when compared to materials of xenogeneic origin. The current study demonstrated that the original ridge profile could not be fully maintained at any time-point in case the ridge was subjected to spontaneous healing (control). These results corroborate with previously published pre-clinical data comparing RP-procedure with spontaneous healing (de Barros et al., 2017, Araujo et al., 2008). In contrast, the horizontal dimension of the ridge remained significantly more stable in both test groups. This led to a

significantly bigger ridge width and subsequently to an improved ridge dimension at sites where RP had been performed. These results are consistent with the previously published data for the volumetric outcomes (Naenni et al., 2017). Furthermore, the difference between the true and the estimated ridge profile (ROI 2 – ROI 1) revealed, for the control group, that the profile was never restored. The grafted groups however, reached positive values at all time-points.

In terms of tissue composition, both test groups showed a similar amount of bone tissue for all three healing periods. At later time-points, these values were higher in all investigated groups. Significant changes were observed between both test groups and the control group at 4 and 8 weeks, but not at 16 weeks. These results are somewhat in contrast with previously published data showing higher amounts of mineralized bone tissue at (xenogeneic) grafted sites (Cardaropoli et al., 2005). On the other hand, similar results were obtained at control sites with 39% bone tissue at sites left for spontaneous healing. Furthermore, after healing periods of 8 and 16 weeks and 3 months respectively, the formation of a hard tissue bridge at the top of the ridge was observed in both studies. This led to a more distinct crestal bone formation resulting in pronounced vertical ridge augmentation in both test groups at 16 weeks in the current study.

When comparing the amount of bone substitute material at the 4-week healing time-point between the test groups, a significantly lesser amount was present in group test1 compared to group test2. Additionally, a considerably higher volume loss was measured for test2 within the first 4 and 8 weeks compared to test1. A part of this volume loss might have been caused by a dislocation of the bone substitute particles used in group test2 rather than having originated from remodeling processes since only slight signs of degradation were observed in the histopathological analysis. Differences, however, leveled out at 16 weeks. The differences between the two test groups might be attributed to the region measured and the different morphological properties of the two materials. The material used in group test1 consisted of PLGA-coated spherical shapes of HA/ß-TCP, whereas the material in group test2 consisted of uncoated, sharp-edged HA/ß-TCP particles. Furthermore, the histological changes at the extraction sites showed no measurable resorption of both alloplastic materials within this study's time-frame. Both bone substitute materials were partly osseointegrated and no considerable

degradation was visible at the surface of the bone substitute materials in both test groups. Similar results regarding conversion were reported in a pre-clinical study, where the biphasic allograft did not undergo marked resorption (Lindhe et al., 2013). Nevertheless, it had allowed for new bone formation, whilst integrating the bone substitute particles within the newly built bone. This again is in congruence with the results of the present study.
Regarding the composition of the tissues measured at the experimental sites, available data reported that the tissues present at an extraction site appeared to be more mature than those present at a surgically created defect of similar dimension (Cardaropoli et al., 2005). In the present study, 50% of the buccal bone wall was surgically removed before performing ridge preservation procedure. Thus, results regarding tissue composition might have been more favorable in undisturbed extraction sockets.

In terms of ridge maintenance, previous pre-clinical studies showed better results if tooth extraction was followed by a ridge preservation procedure compared to spontaneous healing (Araujo et al., 2008, Araujo and Lindhe, 2009, Cardaropoli et al., 2005). Although the dimensions could not be fully maintained, bone substitute materials led to better ridge stability, but seemed to influence remodeling processes. The outcomes of the present study are somewhat in contrast, as the ridge profile even increased in the test groups, whereas it was never restored in the control group. Nevertheless, the use of bone substitute materials in general did seem to disturb natural healing, irrespective of the nature of the graft substitute (xenografts or alloplastic), and did not result in comparable amounts of new bone present as reported after healing periods of 3 months (Cardaropoli et al., 2005) and after 16 weeks in the present study. This seems to be reflected by the fact that a higher amount of (newly formed) bone tissue was observed in the control group where no graft material had been used.
The results of the current study showed a positive effect on ridge stability when using alloplastic materials for ridge preservation procedures. The outcomes of the study can only to some extent be compared with xenogeneic materials since no xenogeneic bone substitute material was used as a control group. Future studies might be directed at investigating the best treatment modality

of the present study compared to xenogeneic bone substitute materials and resorbable collagen membranes, a combination of materials that is frequently used in clinical settings.

In summary, the use of alloplastic bone substitute materials led to better maintenance and even an augmentation of the ridge profile, but decelerated new bone formation. In contrast, sites left for spontaneous healing demonstrated a higher amount of new bone formation, but were never able to restore initial ridge dimensions. Thus, it remains to be investigated whether the histologically observed alterations and remodeling processes may play a crucial role in the clinic.

Conclusions

Alloplastic materials can successfully be used for ridge preservation procedures. Both test materials rendered greater horizontal dimensions and a more favorable maintenance of the ridge profile, but exhibited significantly less new bone formation compared to spontaneous healing.

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Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

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Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Informed consent

For this type of study, formal consent is not required.

Figure legend

Figure 1a

Illustration of the surgical procedures (extraction of the distal root, devitalization, removal of 50% of the buccal bone), that were carried out without raising a flap. (On the first two figures, the buccal bone is not drawn for a better visibility.)

Figure 1b

Illustration of the ridge preservation procedures performed in groups test 1 and test 2. The sites in the control group were left for spontaneous healing.

Figure 2

Histological samples depicting the linear measurements performed regarding quantitative evaluation of the horizontal ridge dimension.
Blue region: Region of interest (ROI 1) = region containing newly formed bone (control group) or bone substitute material and/or new bone (test 1; test 2) corresponding to the ridge profile after the respective healing period.
Yellow lines: horizontal lines used to measure the distance from the defect margin (old bone) to the buccal new bone or bone substitute material at 1mm and 3mm below the lingual bone crest.

Figure 3

Histological samples showing the areas of the respective ridge profile (ROI 1) and the estimated ridge profile (ROI 2).

Figure 4

Diagram depicting the horizontal ridge width at the different time points measured in mm at 1mm and 3mm below the lingual bone crest. *$p<0.05$

Figure 5

Maintenance of ridge profile (ROI1 / ROI2) in % for all investigated groups at 4, 8 and 16 weeks.*$p<0.05$

Figure 6

Histological slides showing the three groups (test 1, test 2, control) at 4, 8 and 16 weeks of healing. a=-old bone; b=new; c=alloplastic bone substitute material (test 1); d= alloplastic bone substitute material (test 2).

Table 1

The table shows the measurements for total horizontal width as well as for bone and bone substitute within ROI 1 (actual ridge profile) in mm after 4 weeks, 8 weeks and 16 weeks healing period;

Test 1: in situ hardening alloplastic bone substitute material (polylactic-co-glycolic acid (PLGA)-coated biphasic calcium phosphate particles consisting of 60% hydroxyapatite (HA) and 40% beta-tricalcium phosphate (ß-TCP));

Test 2: alloplastic bone substitute material (biphasic calcium phosphate consisting: 60% HA and 40% ß-TCP);

Control: spontaneous healing, blood clot.

n = number, Mean = mean value; SD = standard deviation. * $p<0.001$ (Control/Test groups); # $p=0.001$ (Test 1/Control); ° $p=0.007$ (Test 1/Test 2)

Table 2

Table showing the quantitative values in ROI 2 (estimated ridge profile). The data is expressed both in mm2 and in % regarding the respective material (bone tissue, soft tissue and bone substitute material) measured in ROI2 for all three groups. * $p=0.001$

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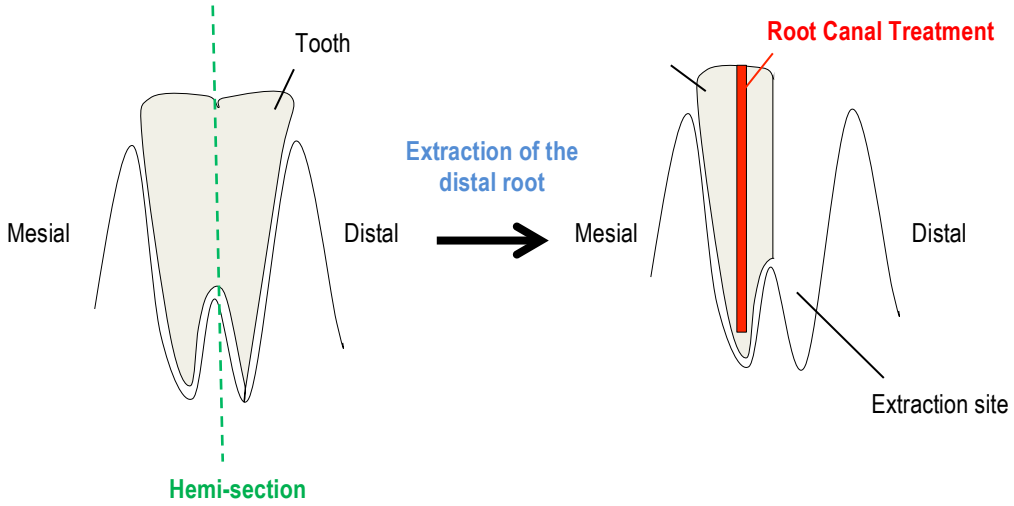
			Total horizontal width (mm) (bone, bone substitute, soft tissue)		**Horizontal width bone tissue (mm)**		**Horizontal width bone substitute (mm)**	
			1mm	**3mm**	**1mm**	**3mm**	**1mm**	**3mm**
4 weeks	**Test1** (n=5)	**Mean**	**2.6**	**3.4**	**0.2**	**0.3**	**0.8**	**1.0**
		SD	*0.5*	*0.4*	*0.3*	*0.3*	*0.5*	*1.0*
	Test2 (n=6)	**Mean**	**3.2**	**3.3**	**0.0**	**0.1**	**1.0**	**0.9**
		SD	*0.6*	*0.4*	*0.1*	*0.1*	*0.2*	*0.2*
	Control (n=4)	**Mean**	**0.8 ***	**1.5***	**0.4***	**0.9***	**0.0**	**0.0**
		SD	*0.7*	*0.6*	*0.3*	*0.3*	-	-
8 weeks	**Test1** (n=5)	Mean	**2.4**	**2.7#**	**0.3**	**0.8**	**0.8**	**1.1**
		SD	*0.3*	*0.6*	*0.3*	*0.3*	*0.2*	*0.8*
	Test2 (n=7)	Mean	**3.0**	**3.4**	**0.0**	**0.4**	**0.7**	**0.8**
		SD	*0.4*	*0.7*	*0.1*	*0.4*	*0.2*	*0.4*
	Control (n=5)	Mean	**1.1#**	**1.5#**	**0.9#**	**0.9**	**0.0**	**0.0**
		SD	*0.3*	*0.7*	*0.3*	*0.5*	-	-
16 weeks	**Test1** (n=4)	Mean	**3.0**	**3.3**	**0.5**	**0.9**	**1.3**	**1.1**
		SD	*0.4*	*0.6*	*0.2*	*0.2*	*0.4*	*0.5*
	Test2 (n=6)	Mean	**3.1**	**3.5**	**0.8**	**0.7**	**0.5**	**0.8**
		SD	*0.6*	*0.7*	*0.6*	*0.3*	*0.1*	*0.2*
	Control (n=5)	Mean	**1.0 °**	**1.7**	**0.8°**	**1.3°**	**0.0**	**0.0**
		SD	*0.4*	*0.1*	*0.4*	*0.2*	-	-

* $p<0.005$ (Control/Test groups)
# $p\leq0.001$ (Control/Test groups)
° $p\leq0.0001$ (Test1/Test2)

			Quantitative values in ROI 2 (estimated ridge profile) *Values expressed in %*		
			Bone tissue	**Soft tissue**	**Bone substitute material**
4 weeks	**Test1** (n=5)	**Mean**	**6.6** *	**56.3**	**37.1**
		SD	*5.8*	*17.9*	*20.8*
	Test2 (n=6)	**Mean**	**3.6** *	**51.1**	**45.3**
		SD	*2.1*	*7.0*	*8.2*
	Control (n=4)	**Mean**	**50.7** *	**49.4**	**0.0**
		SD	*8.7*	*8.7*	*0.0*
8 weeks	**Test1** (n=5)	**Mean**	**17.2** +	**47.1**	**35.7**
		SD	*2.9*	*4.1*	*3.5*
	Test2 (n=7)	**Mean**	**7.8** +	**59.8**	**32.4**
		SD	*8.4*	*8.4*	*12.2*
	Control (n=6)	**Mean**	**67.7** +	**32.3**	**0.0**
		SD	*12.2*	*12.2*	*0.0*
16 weeks	**Test1** (n=4)	**Mean**	**25.6** ♀	**33.2**	**41.3**
		SD	*6.8*	*3.5*	*8.4*
	Test2 (n=6)	**Mean**	**24.9** ♀	**48.8**	**26.4**
		SD	*12.8*	*12.9*	*4.2*
	Control (n=5)	**Mean**	**77.0** ♀	**23.0**	**0.0**
		SD	*9.8*	*9.8*	*0.0*

* p=0.0060
+ p<0.0001
♀ p=0.0002

Fig.1a


Tooth
Mesial
Distal
Hemi-section
Extraction of the distal root
Root Canal Treatment
Mesial
Distal
Extraction site

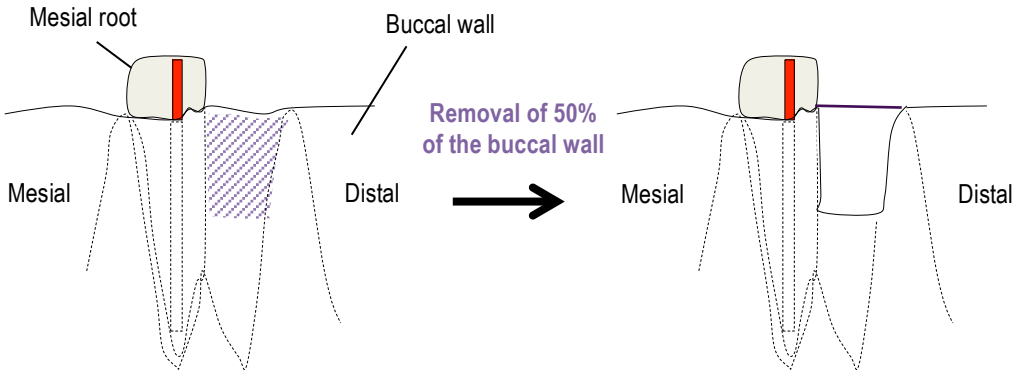
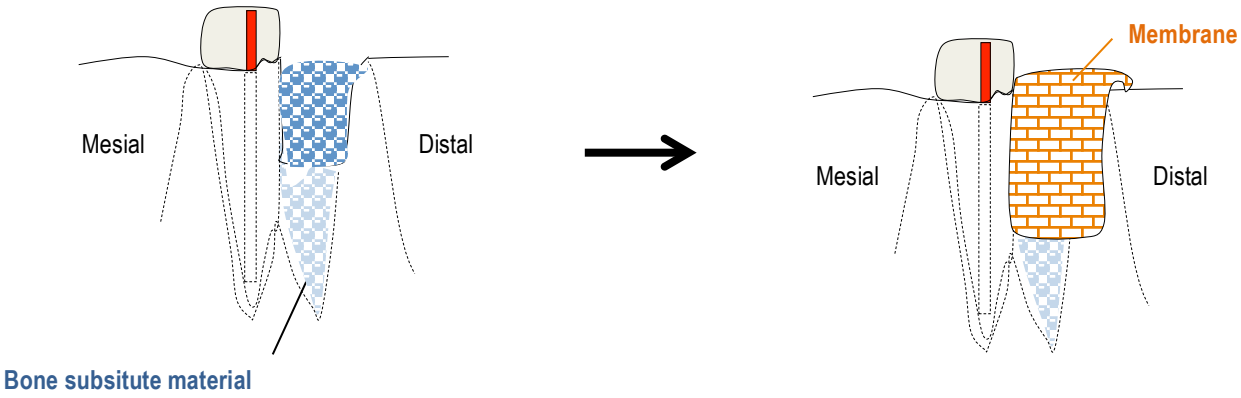
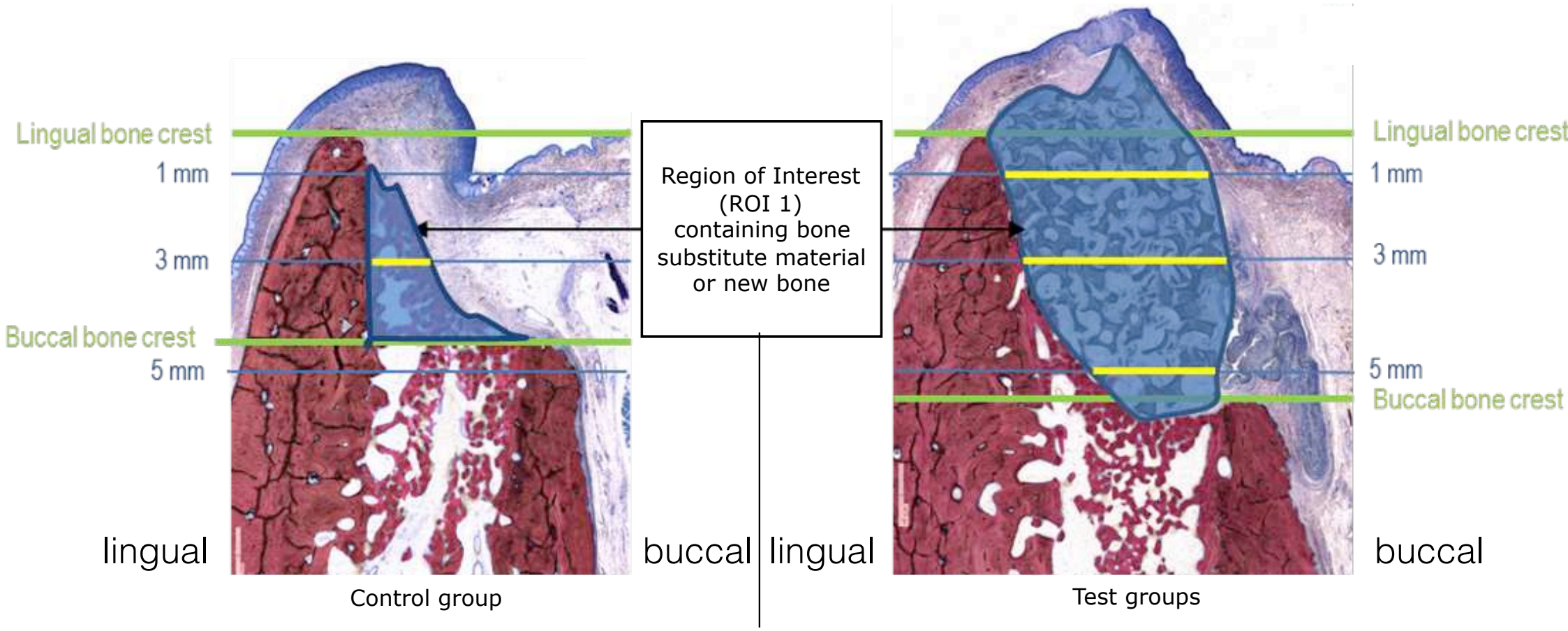
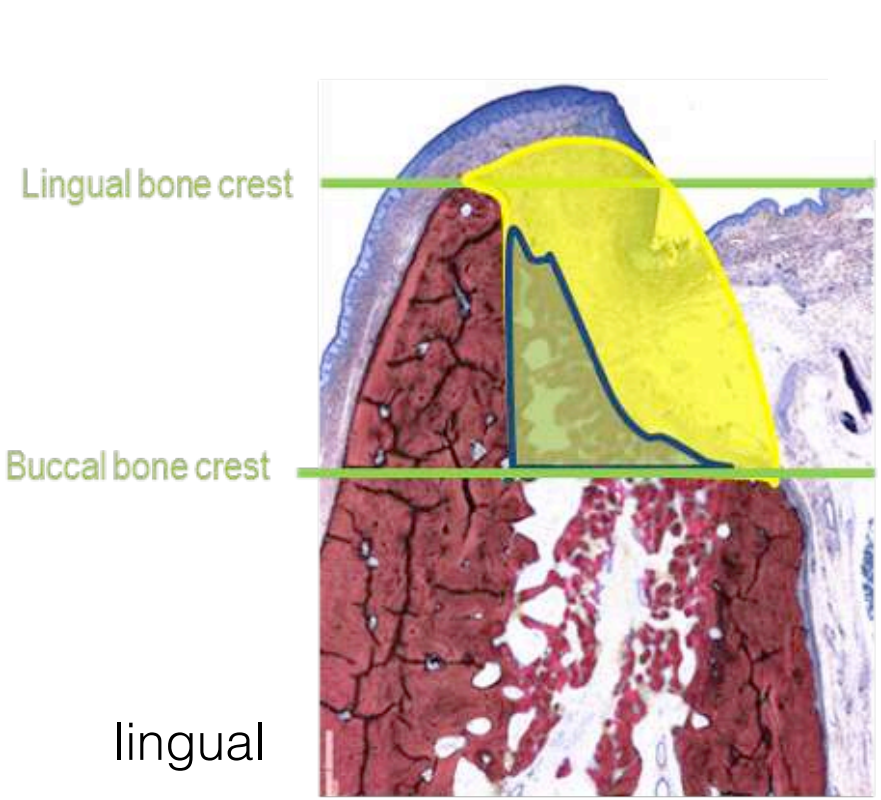

Mesial root
Buccal wall
Mesial
Distal
Removal of 50% of the buccal wall
Mesial
Distal

Fig.1b

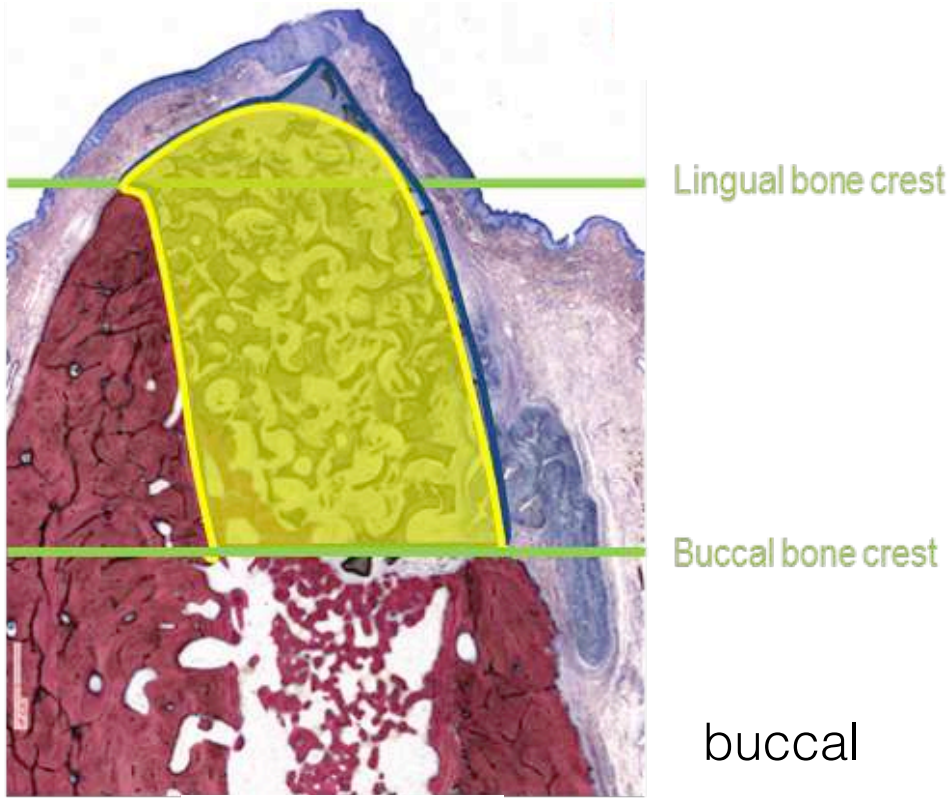

Mesial
Distal
Bone subsitute material
Membrane
Mesial
Distal



Bucco-lingual cross-section of histological slide at the center of the defect



Control group

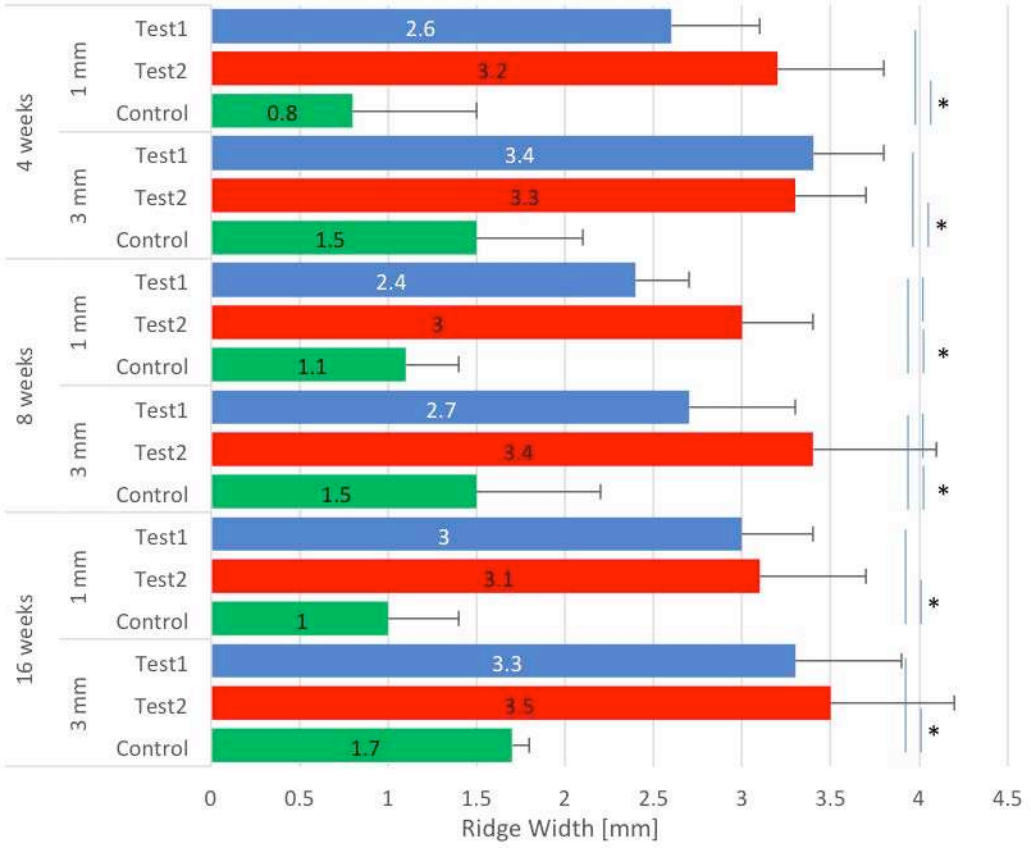


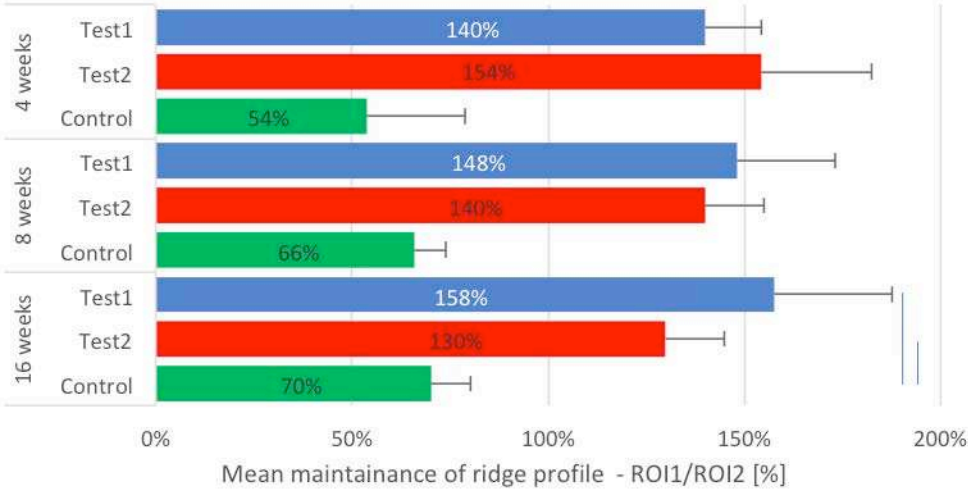
Test groups

ROI 2 = Estimated ridge profile: Area equivalent to 100% of the original ridge profile

ROI 1 = Ridge profile including:
- new bone (control group)
- bone subsitute material and/or new bone (test groups)

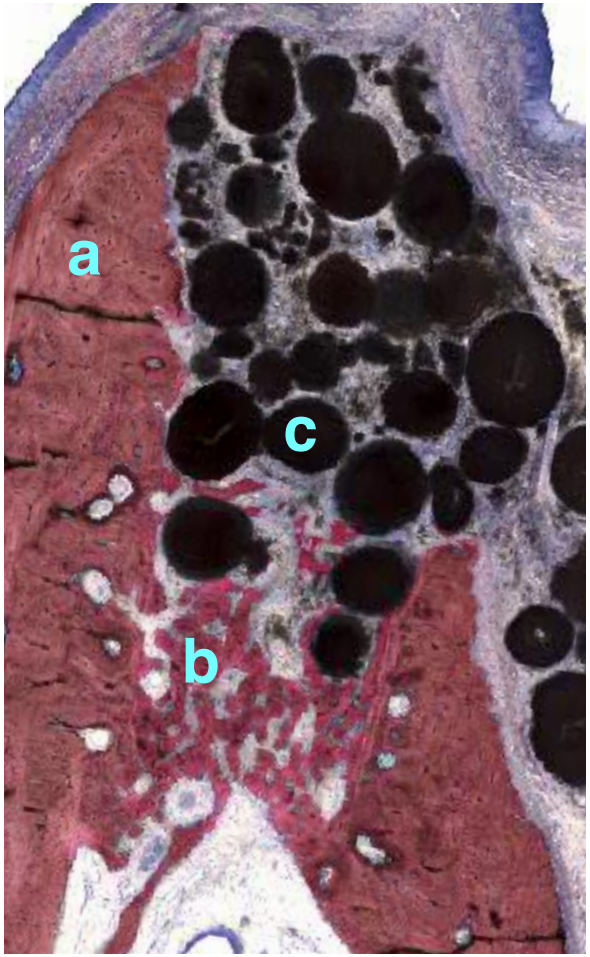
Bucco-lingual cross-section of histological slide at the center of the defect


4 weeks
8 weeks
16 weeks
1 mm
3 mm
Test1
Test2
Control
2.6
3.2
0.8
3.4
3.3
1.5
2.4
3
1.1
2.7
3.4
1.5
3
3.1
1
3.3
3.5
1.7
*
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
Ridge Width [mm]

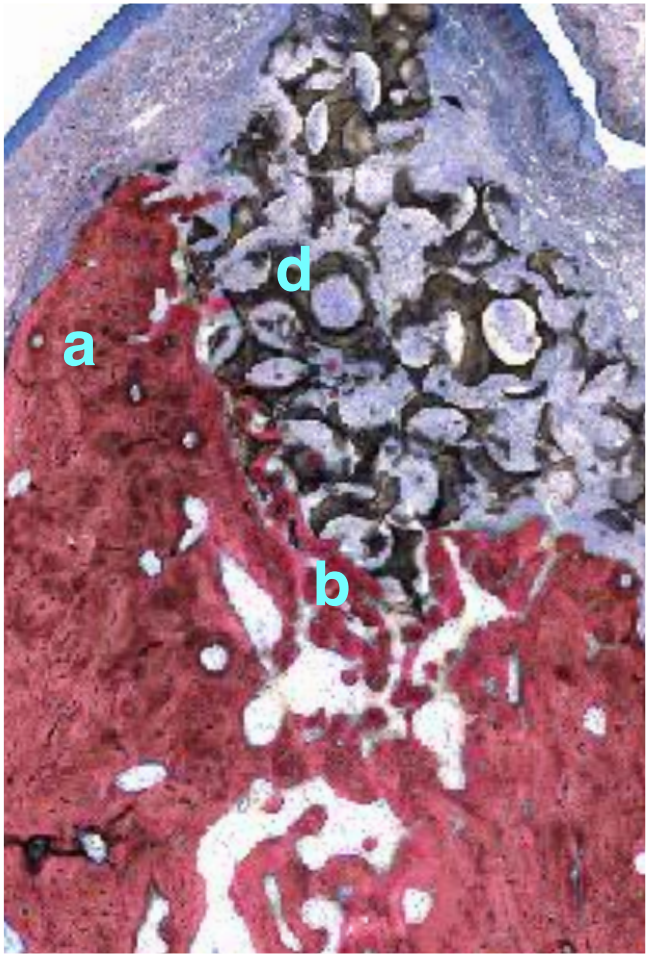

4 weeks
Test1
Test2
Control
140%
154%
54%
8 weeks
Test1
Test2
Control
148%
140%
66%
16 weeks
Test1
Test2
Control
158%
130%
70%
0%
50%
100%
150%
200%
Mean maintainance of ridge profile - ROI1/ROI2 [%]

4 weeks

control

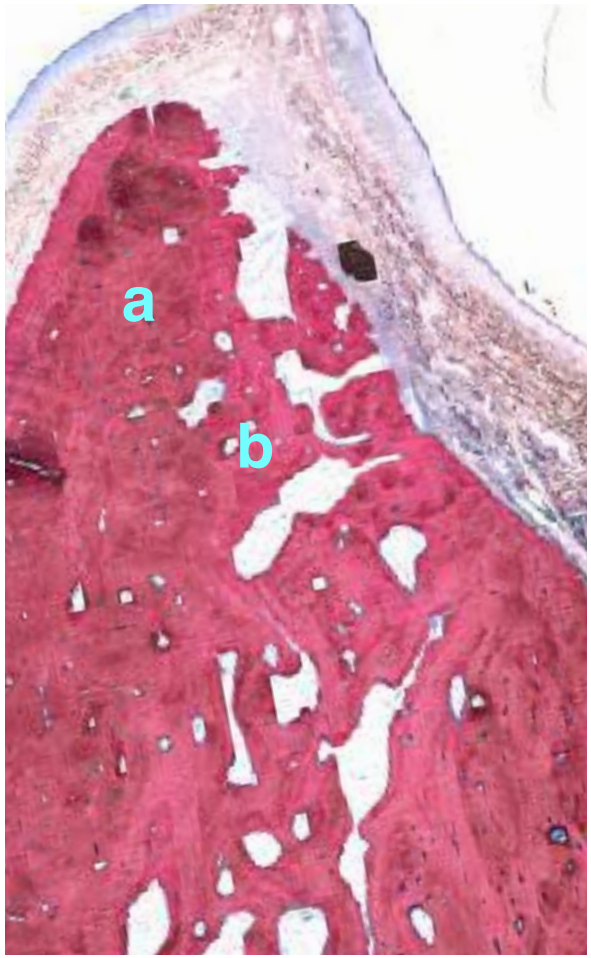


test 1

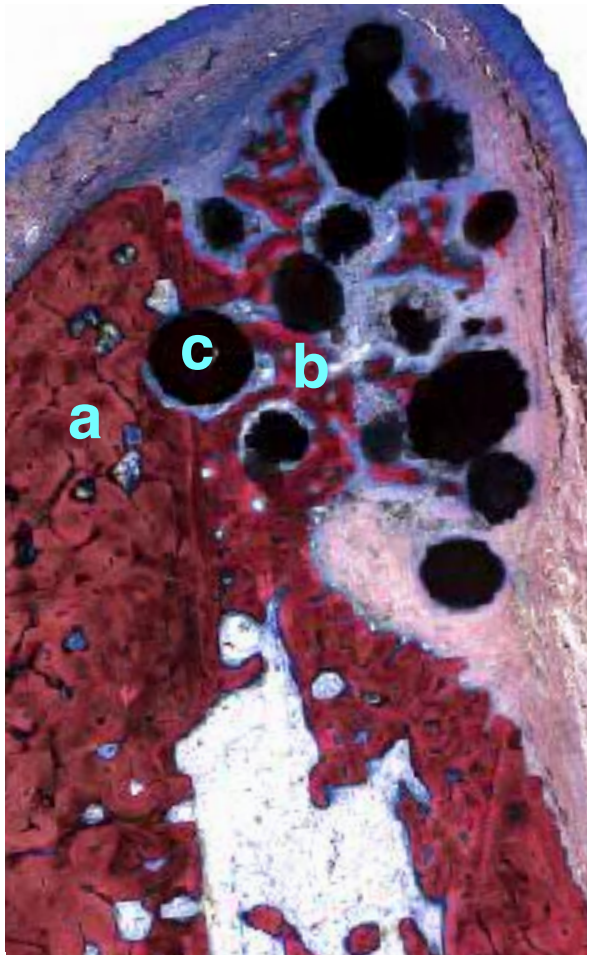


test 2

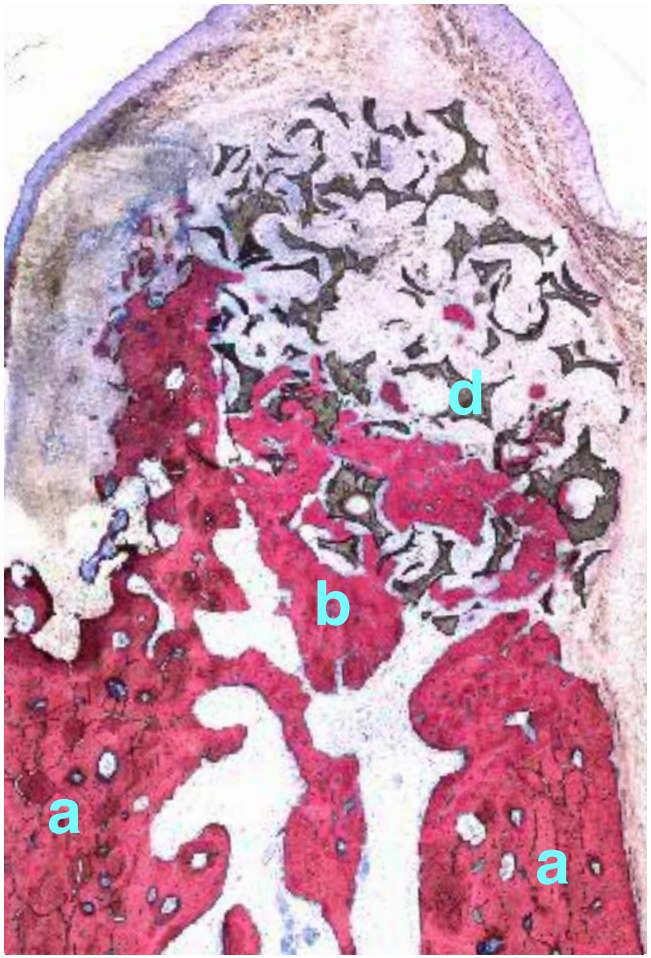
8 weeks



control

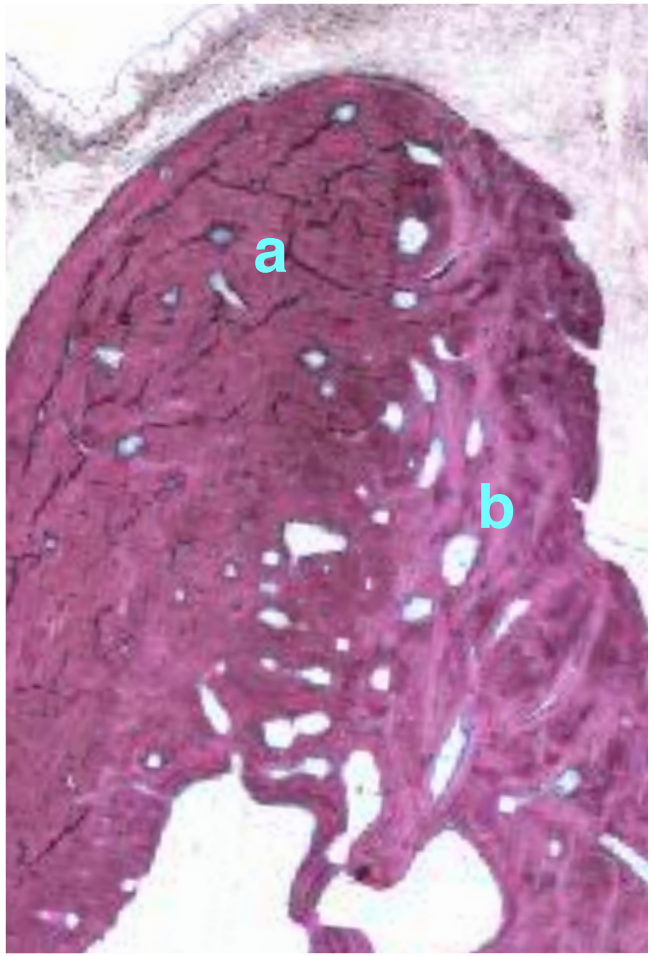


test 1

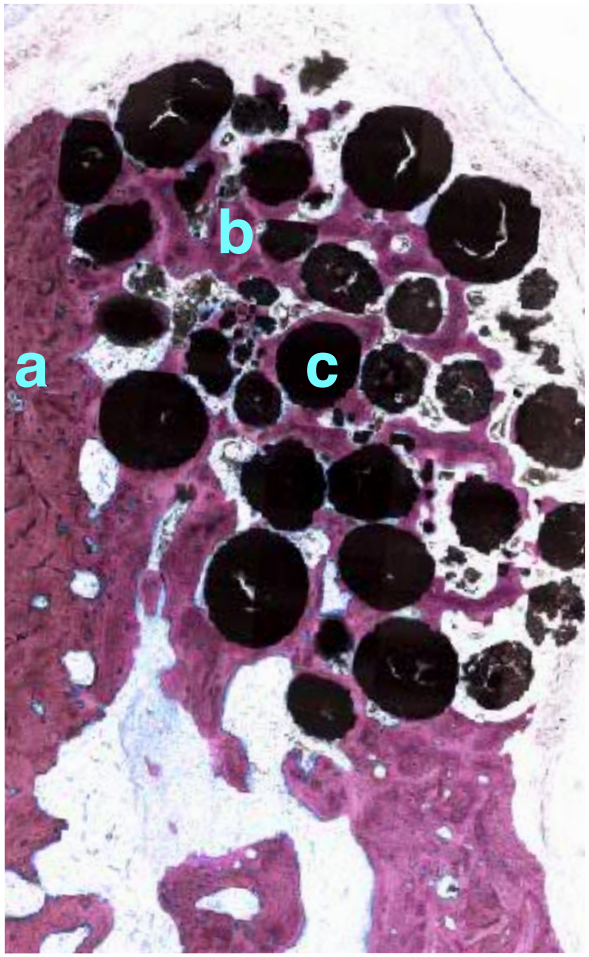


test 2

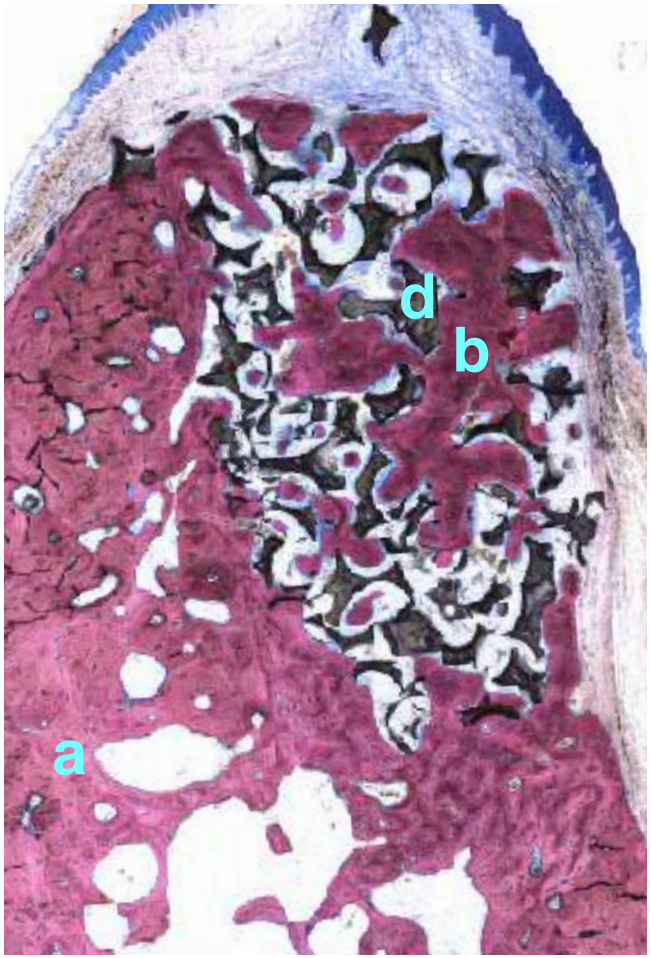
16 weeks



control



test 1



test 2